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MAIZE GENES INDUCED BY HERBIVORY AND VOLICITIN

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Abstract—In crop plants, both mechanical damage and insect attack trigger rapid changes in gene transcription. We investigated whether insect herbivory differs from a general wound response, and if so, is the induction specific to the pest/host plant interaction? Herbivory by beet armyworm (BAW; *Spodoptera exigua*) caterpillars on maize results in a unique pattern of volatile compounds not triggered by wounding alone that attracts the generalist parasitoid *Cotesia marginiventris*. Caterpillar-induced volatile emission can be mimicked when a component of the BAW oral secretions (*N*-(17-hydroxylinolenoyl)-L-glutamine) termed volicitin, is applied to wounded leaves. We identified genes that are affected by BAW feeding by comparing volicitin treatment with wounding alone. We compared cDNAs from these two populations by isolating genes from a subtractive library and using reverse northerns. Virtual northern blots confirmed these results and further showed that BAW infestation affected the expression of these genes. In some cases, BAW feeding inhibited the expression of volicitin-induced genes, suggesting the role of additional bioactive components in caterpillar regurgitate. Transcripts involved in volatile production are increased by volicitin and BAW infestation treatments, and are also detectable at low levels in mechanically wounded leaves. Finally, we identified three new sesquiterpene cyclase genes that are induced by volicitin.

Key Words—Insect herbivory, maize, plant–insect interaction, volicitin.

INTRODUCTION

Herbivory induces an array of genes, including those responsible for direct and indirect defense responses. Direct defenses are commonly metabolites that interfere with insect feeding and nutritions (Kessler and Baldwin, 2002), such as proteinase inhibitors that inactivate digestive enzymes, and polyphenol oxidase, which crosslinks plant proteins making them less nutritions. Indirect defenses

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occur when products from the infested plant attract natural enemies of the attacking insect (Dicke, 1999). For example, infestation of maize with beet armyworm (BAW; *Spodoptera exigua*) causes the production of volatile compounds that attract the generalist parasitoid *Cotesia marginiventris* to the BAW larval host (Turlings et al., 1990). After oviposition, the parasitoids develop inside the lepidopteran larvae, killing them, reducing the level of herbivory, and preventing further pest reproduction (Hoballah and Turlings, 1999). A wide range of plant species such as apple, lima bean, and cotton produce similar volatile compounds in response to insect herbivory (Pare and Tumlinson, 1999).

Induced volatile emission following mechanical damage and BAW herbivory differ greatly, and enables efficient searching by parasitoids. However, the profile of volatiles produced by the infested leaf can be mimicked if a mechanically damaged leaf is treated with volicitin, a fatty acid-amino acid conjugate (FAC) in BAW regurgitant (Alborn et al., 1997). In addition to volicitin, several classes of elicitors from insect oral secretions have been described. A β-glucosidase from *Pieris brassicae* (cabbage-white butterfly) (Mattiacci et al., 1995), Glc oxidase from *Helicoverpa zea* (corn earworm) (Musser et al., 2002), and additional FACs from lepidopteran species (Pohnert et al., 1999; Halitschke et al., 2001) all alter plant responses when applied to a wounded leaf.

Evidence exists predicting that the increase of certain volatiles is a result of *de novo* protein synthesis (Pare and Tumlinson, 1997), suggesting an increase in transcript levels of genes associated with this process. In fact, the gene *Igl*, which encodes an enzyme that produces the volatile indole, is induced quickly upon addition of volicitin to wounded tissue (Frey et al., 2000). The gene products of *TPS1* and *Stc1* produce volatiles sesquiterpene (Shen et al., 2000; Schnee et al., 2002), and the steady state level of the transcript is higher following insect herbivory and elicitor application.

To clarify further the transcriptional basis of insect-specific defense responses, we set out to isolate genes that are differentially affected by insect elicitors. This was accomplished by the production of a subtractive cDNA library in which RNA from mechanically damaged tissue was subtracted from RNA that had been extracted from tissue that had been wounded and treated with volicitin. By using excessive wound-induced RNA, genes that are particularly sensitive to herbivore elicitors rather than mechanical damage, a non-specific component of insect herbivory, are preferentially cloned. Generally we found that genes involved in volatile production were induced and isolated three putative sesquiterpene cyclase genes.

METHODS AND MATERIALS

Plant Growth Condition. Seeds of *Zea mays* cv Delprim were acquired from Delley Seeds (Delley, Switzerland). Plants were germinated in vermiculite,

grown for 6 d, and then transferred to hydroponic containers (see Schmelz et al., 2001). The hydroponic solution was complete except for limiting levels of NO_3^- (0.2 mM) as described in Schmelz et al. (2003a). These low N conditions increase plant sensitivity and induced volatile responses to volicitin.

Physical and Chemical Leaf Treatments. For mechanical damage treatments, each of the oldest three leaves of individual plants received two superficial damage sites by using a razor to scratch the abaxial surface of the leaves perpendicular to but not including the midrib vasculature. The mechanical damage sites (normally 2 × 10 mm) were approximately equidistant between the base and tip of the leaf but laterally staggered by 2 cm with one on each side of the midrib. This treatment disrupted the waxy cuticle and epidermal cells and allowed applied buffer solutions to cling to the leaf surface. A total of 10 μl of 50 mM sodium phosphate (pH = 8.0) buffer were distributed evenly between all mechanical damage sites on each plant immediately after wounding. The quantity of volicitin dissolved in buffer and applied to each plant was 5 nmol. Leaf treatments were performed immediately before the end of the photophase (6 p.m.). In corn, BAW herbivory induces ethylene emission, but mechanical damage volicitin treatments do not (Schmelz et al., 2003b). To better mimic insect attack, both wounded and wound + volicitin treated plants were additionally exposed to 50 nl L^{-1} ethylene during the overnight incubation period as described in Schmelz et al. (2003a). Intact plants were placed in sealed 7 L cylindrical Plexiglas™ chambers (12 × 62 cm) fitted with rubber septa for the introduction of ethylene. Ethylene (Scotty® II Gases, Alltech, Deerfield, IL) addition was always performed immediately after elicitor treatments.

Analyses of Volatiles. Collection and gas chromatography analysis of volatiles were performed as described in Schmelz et al. (2001). Plant volatiles were collected for 1 hr at 12 hr after treatment. This period corresponds with maximal volatile emission (Schmelz et al., 2001, 2003c). The three major insect-induced sesquiterpenes (cs) in maize (var Delprim) are β-caryophyllene, (*E*)-α-bergamotene, and (*E*)-β-farnesene.

RNA Isolation and Subtractive Library Production. RNA was isolated from tissue frozen in liquid nitrogen and ground to a fine powder using the procedure described in Chang et al. (1993). Maize tissue was extracted with vigorous shaking in a buffer preheated to 65°C containing 2% CTAB, 2% polyvinylpyrrolidone, 100 mM Tris pH 8.0, 25 mM EDTA, 2 M NaCl, 0.5 g/l spermidine, and 2% β-mercaptoethanol. This mixture was chloroform extracted. The aqueous layer was precipitated at 4°C overnight in an ice water bath with 1/4 volume of 10 M LiCl. The pellet was collected by centrifugation at 11,950 × g for 20 min and resuspended in 1.0 M NaCl, 0.5% SDS, 10 M Tris pH 8.0, and 1 M EDTA. RNA was extracted once with chloroform and precipitated in two volumes of 95% ethanol.

In order to preferentially clone volicitin-induced genes, a subtractive cDNA library was produced using the PCR-Select cDNA subtraction kit[2] from Clontech (Palo Alto, CA) following the procedure outlined by Diatchenko et al. (1996). Double stranded cDNA was synthesized using a SMART™ PCR cDNA Synthesis kit from Clontech. An excess of cDNA from wounded material was hybridized to cDNA from wounded + volicitin-treated material, which enriches volicitin-induced cDNAs. The resulting cDNAs were cloned into pGem-T using Vector system I from Promega (Madison, WI).

Differential Screening of the Subtracted Library. The cDNA clones were amplified by PCR with kit-specific primers (nested primer 1 and nested primer 2R), and the DNA from each clone was mixed with an equal volume of 0.6 N NaOH and spotted onto nylon membrane in a 96-well format. The blots were neutralized in 0.5 M Tris–HCl (pH 7.5) and washed in water. The DNA was baked onto the blots for 2 hr in a 70°C oven. The dot blots were hybridized with either ^{32}P random labeled volicitin-induced enriched cDNA or wound-induced enriched cDNA probe following a protocol for screening a cDNA subtractive library described in the PCR-select differential screening kit manual from Clontech. The volicitin-induced enriched probe was prepared by hybridizing an excess of wound-induced cDNA from volicitin-induced cDNA, thus enhancing volicitin-induced genes. The wound-induced enriched probe was prepared by hybridizing an excess of volicitin-induced cDNA to wound-induced cDNA, enhancing genes that were induced differentially in the wounded tissue. The probes were labeled with ^{32}P using the Prime-It II random primer labeling kit from Stratagene (Cedar Creek, TX).

Virtual Northern Blots and Generation of Longer cDNAs of Sesquiterpene Synthases. Virtual northerns were performed as described in Endege et al. (1999) using a protocol included in the cDNA synthesis kit from Clontech. This procedure is more sensitive than a standard Northern in that it uses cDNA rather than RNA. cDNA from different RNA samples was made using a poly-T primer along with annealing of a 5′ universal primer for PCR amplification of the cDNA. The amplified cDNA was loaded and run on an agarose gel, and capillary transfer of the cDNA was performed (Sambrook et al., 1989). Nested primers 1 and R2 were used to amplify selected clones to be labeled with ^{32}P using the Prime-It II random primer labeling kit from Stratagene. Virtual northern blots were hybridized and washed using the ExpressHyb hybridization solution from Clontech.

Since the initial clones were not full length, 3′ and 5′ ends of sesquiterpene synthase clones were generated using 5′ and 3′ RACE kits from Invitrogen (Carlsbad, CA) following a protocol outlined by Frohman (1990). Using a

[2]Disclaimer: Mentioning of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the USDA and does not imply its approval to the exclusion of other products that may also be suitable.

gene-specific primer that binds approximately 300 bp from the known 5′ end of the RNA, cDNA was produced using reverse transcriptase. The RNA was then degraded with RNase, and the single stranded cDNA was tailed with deoxycytidine using terminal deoxynuceotidyl transferase. The tailed cDNA was made double stranded by PCR amplification using a kit-specific primer that bound to the polyC tail and a gene-specific primer that bound 3′ of the first gene specific primer site. Finally, the cDNA was amplified and made more specific by PCR amplification using a third gene-specific primer and a kit-specific primer that binds to the 5′ end. The 3′ ends of clones were produced by making single stranded RNA using an oligo(dT) kit specific primer. The RNA was degraded with RNase H, and the cDNA was made double stranded by PCR amplification using a gene-specific primer and a primer that binds to the oligo(dT) kit-specific primer. The PCR reaction was run on a 1% agarose gel in TBE, and a pipette tip was used to isolate a piece of the ethidium bromide-stained band. The gel piece was used in a final PCR reaction using a second gene-specific primer and a kit-specific primer that binds to the 3′ end. The cDNAs were cloned into pGem-T using Vector system I from Promega (Madison, WI).

Sequence Analysis. Clones that were either induced or inhibited by the addition of volicitin to the tissue were selected and sequenced using Bigdye terminator sequencing by Applied Biosystems (Foster City, CA) using a 3100 ABi Prism sequencer. DNA sequences were analyzed using DNASTAR. Sequences were compared with the database at the National Center for Biotechnology Information using tBLASTx and BLASTx (Altschul et al., 1997). A phylogenetic tree for the cloned sesquiterpene synthases was generated using neighbor joining and distance programs of PAUP (Swofford, 1998. Version 4. Sinauer Associates, Sunderland, MA).

RESULTS AND DISCUSSION

Volatile Production. Addition of volicitin to wounded leaves led to an increase in emission of indole and sesquiterpene volatiles (Figure 1). The predominant sesquiterpenes in BAW-infested and volicitin-treated maize (cv Delprim), include β-caryophyllene, (E)-α-bergamotene, and (E)-β-farnesene. Thus, an upregulation of genes involved in sesquiterpene synthesis was predicted. Although measurement of volatiles occurred 12 hr after mechanical damage and volicitin addition, plants were harvested after only 3 hr for RNA isolation, since we assumed that maximal mRNA production of sesquiterpene genes would precede the maximal production of volatiles. Total RNA was extracted (Chang et al., 1993) and a subtracted cDNA library was produced (see "Methods and Materials"). To confirm differential expression of the isolated cDNAs, individual clones were hybridized with ^{32}P labeled cDNA from forward-subtracted and reverse-subtracted probes. Forward-subtracted probe is enhanced for volicitin-induced cDNAs, while the

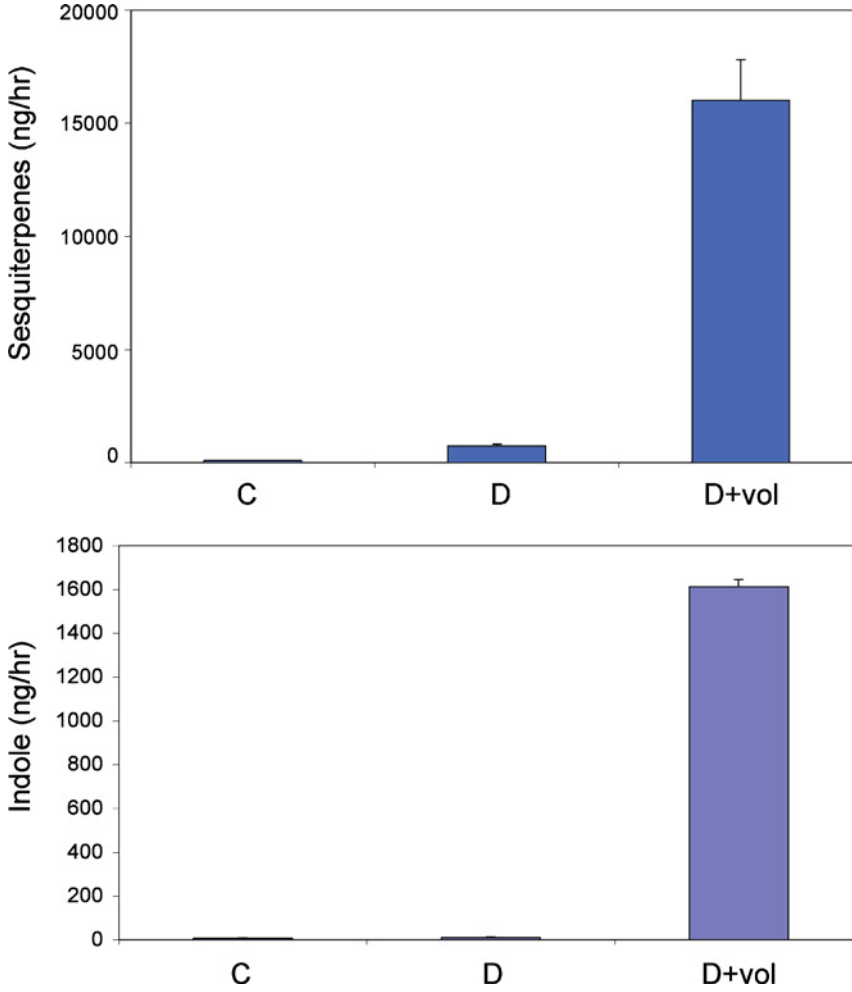

FIG. 1. Sesquiterpenes and indole are induced by volcitin treatment. Five nanomoles of volicitin was added to mechanically damaged leaves. C, Control; D, damaged; D+Vol, damage plus 5 nmol volicitin.

reverse-subtracted probe is enhanced for wound-induced cDNAs. Measurement of the radioactivity hybridizing to the individual clones using a phosphorimager allowed calculation of the ratio of the forward-subtracted cDNA divided by the reverse-subtracted cDNA. Values over 1 suggest that the clone is more abundant in the volicitin-treated sample, while values under 1 imply that the clone is more abundant in the wounded sample.

TABLE 1. GENES FOUND TO BE DIFFERENTIALLY EXPRESSED BY VOLICITIN TREATMENT

Ratio	Gene	Homology (tblastX)	Accession #	*E* value
>2	7	*Oryza sativa* putative sesquiterpene cyclase 1 (blastX)	BAB63870	8.00E–26
>2	8	No homology		
>2	9	*Oryza sativa* GDP dissociation inhibitor protein OsGDI1	AF016896	7.00E–59
>2	14	*Z. mays* CL39675_1 mRNA sequence	AY110070	3.00E–17
>2	15	*Z. mays* PCO129667 mRNA sequence	AY106046	1.00E–142
3.4	16	Salicylic acid carboxyl methyltransferase	AB049752	9.00E–75
9.2	18	*Z. mays* sesquiterpene cyclase 1 mRNA, Stc1	AF296122	2.00E–50
3.7	22	*Lycopersicon esculentum* GcpE mRNA	AF435086	0
3.4	24	*Lycopersicon esculentum* Pto kinase interactor 1	U28007	1.00E–163
0.3	25	*Arabidopsis thaliana* metalloprotease -related	NM_112804	1.00E–118
3.6	26	*Oryza sativa* clone OJ991214_12	AL606453	4.00E–44
3.3	28	Oryza sativa methylthioadenosine/S-adenosyl homocysteine nucleosidase	AF458088	4.00E–82
>2	29	*Oryza sativa* putative sesquiterpene cyclase 1	NM_183909	3.00E–42

Note. The ratio refers to the amount of volicitin-induced cDNA that hybridizes divided by the amount of wound induced cDNA that hybridizes to the clones. Unless otherwise noted, the tBlastX program was used to determine homologies and *E* values. Accession numbers are for the genes homologous to the maize genes.

Differential Gene Expression. Sixty-three differentially expressed clones were sequenced and organized into 27 genes (Table 1). Six of the genes had a ratio below 1 and 5 of these are associated with the chloroplast genome, suggesting that plastid-associated genes might be inhibited by volicitin treatment. However, when the chloroplast-associated genes were used as probes on virtual northern blots, they hybridized to numerous bands, and due to the complexity of the banding patterns it was not possible to confirm whether they are differentially expressed (data not shown). The complexity of the banding patterns was probably due to the considerable processing events typical of these polycistronic chloroplast messages (Buchanan et al., 2000). The cDNA was initially produced from total RNA using a primer with $T_{(30)}$ near the 3′ end, so it was initially surprising to find clones of chloroplast mRNAs since poly A+mRNA is not usually associated with chloroplast transcripts. However, messages containing short oligo A+runs have been noted in chloroplast genes (Grierson and Covey, 1984). In addition, chloroplast messages can have poly(A)-rich sequences added post-transcriptionally, which

make them particularly unstable and destined for degradation (Lisitsky et al., 1996). These two explanations may help to explain why six genes have sequences associated with the chloroplast genome. Considering the large amount of chloroplasts in leaf tissue, chloroplast RNA should represent a large population of the mRNA. Since we could not confirm using northerns whether these genes were differentially expressed, they were not included in Table 1.

Virtual northerns, which use cDNA rather than RNA, were used to confirm differential expression and to test whether BAW feeding induces the expression of these clones (Figure 2). Clones from 11 genes were examined on virtual northerns. Gene13 was below the detection limit of the virtual northern blot. Wounding induced the expression of nine of the genes (9, 18, 28, 15, 16, 26, 7, 29, and 24 see Figure 2), and volicitin enhanced the expression of RNA compared to the wounded sample in seven of them (18, 22, 16, 26, 7, 29, and 24). Leaves were subjected to BAW feeding for 6 or 18 hr; the expression of transcripts for these genes was induced in only four cases (18, 25, 7, 29). In five of the clones, less RNA was present in the BAW-infested sample compared to the control (9, 28, 15, 26, and 24). Consequently, four genes were induced by both volicitin and BAW feeding (18, 16, 7, and 29), while two genes were induced by volicitin but inhibited by BAW infestation (26 and 24). Finally, four genes were induced by wounding but inhibited by BAW feeding (9, 15, 26, and 24). Finding two genes that were induced by volicitin treatment but inhibited by BAW infestation was unexpected. Perhaps there is an additional component of BAW saliva that causes this inhibition of these genes. Indeed, Halitschke et al. (2003) found that four clones that were down regulated by regurgitant were induced by a FAC isolated from that regurgitant. In conclusion, although seven of the genes were induced by volicitin treatment, RNA was also found in the wounded sample. Consequently, none of the genes were specifically induced by volicitin.

Two genes may encode defense-signaling proteins. A rab-specific GDP-dissociation inhibitor (rab-GDI) from rice is similar to gene 9. The rice protein, found in fungal-elicitor- or salicylic acid-induced suspension cells, is involved in membrane trafficking, and is thought to be associated with defense signal transduction (Kim et al., 1999). This particular gene was only wound induced and was inhibited by BAW feeding (Figure 2), suggesting that this pathogen-related gene is turned off by insect infestation. Gene 24 encodes a protein similar to tomato Pto kinase interactor 1. This is a serine/threonine kinase that is phosphorylated by Pto. Both of these genes are part of the signaling cascade leading to the hypersensitive reaction in tomato caused by an interaction with bacterial speck (Zhou et al., 1995). Although this gene was induced by volicitin, it was also not on during BAW feeding (Figure 2). Another component of the BAW saliva may help to differentiate this pathogen-associated gene.

Gene 28 is similar to a putative methythioadenosine/*S*-adenosyl homocysteine nucleosidase from rice. This particular enzyme has been characterized in

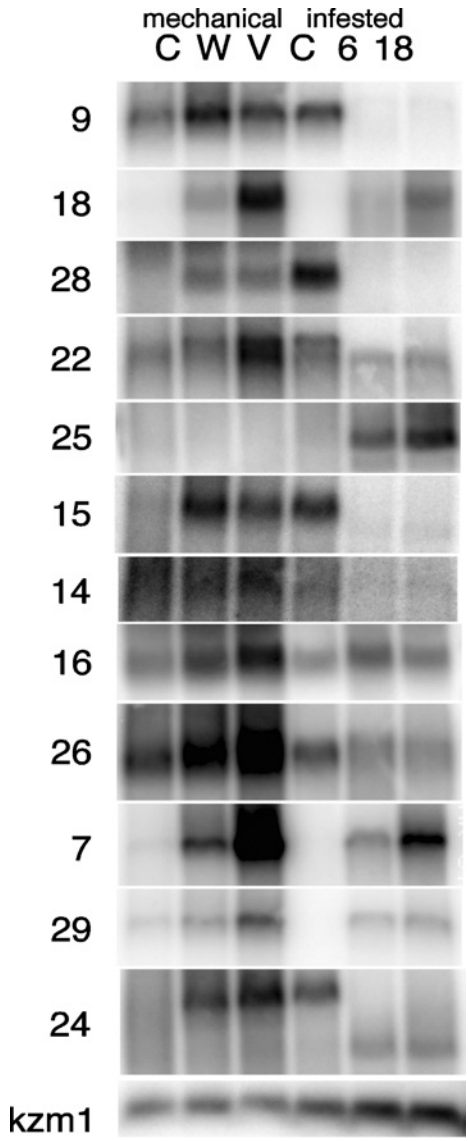

FIG. 2. Volicitin treatment and BAW feeding affected the levels of transcript present in maize leaves. cDNA from the following plant material was probed with various genes identified in Table 1. C, Control; W, wounded; V, wounded plus 5 nmol volicitin was added to leaves that were harvested after 3 hr. Maize leaves were (C) uninfested for 6 hr, or infested with BAW for 6 or 18 hr. *kzm1* constitutively expressed potassium channel gene from maize.

bacteria and purified from plants, where it hydrolyzes the glycosidic bond of 5'-deoxy-5'methyladenosine and *S*-adenosylhomocysteine to produce adenine and methylthioribose or *S*-ribosyl-homocysteine (Cornell et al., 1996). In bacteria, it is believed to have a role in salvaging methionine and adenine (Cornell and Riscoe, 1998). This particular gene was wound induced, while it was inhibited by BAW feeding (Figure 2). It is difficult to speculate about what this may mean considering that little is known about the function of this gene in plants.

Five genes are most likely related to the synthesis of volatile compounds. Monoterpenes (C10) and diterpenes (C20) are produced in the plastid, while sesquiterpenes (C15) are created in the cytosol. Two separate pathways are responsible for these compounds, which use the precursors dimethylallyl diphosphate (DMADP) and isopentyl diphosphate (IPP) (Aubourg et al., 2002). Three different genes (7, 18, and 29) have similarity to sesquiterpene cyclase genes responsible for the synthesis of sesquiterpenes. The three genes are most similar to three different genes in the database (Table 1), suggesting that they encode enzymes producing different compounds. An alignment of the sesquiterpene cyclase genes is shown in Figure 3. The three genes are compared with putative genes from *Arabidopsis*, and the two genes that have been characterized in maize (*stc1* and *tps1*). The *tps1* gene encodes an enzyme that produces $(E) - \beta$-Farnesene, a major component of the volatiles found in volicitin-induced Delprim maize. A 250 amino acid section of the coding region was compared with these genes. A phylogenetic tree of the sesquiterpene cyclases is shown in Figure 4. There was 47.9% identity between genes 7 and 29. There was only 14% and 12.8% identity between genes 18 and 7 or 29, respectively. Genes 7, 18, and 29 had 9%, 22.4%, and 8.6% identity with *tps1*, while they had 12.8%, 49.0%, and 14.0% identity with *stc1*. Clearly, gene 18 is most similar to *stc1*, while genes 7 and 29 are most similar to each other. This similarity is examined further with the phylogenetic tree in Figure 4. Bootstrap analysis is shown at the nodes, and is a measurement of the percent reliability that the branches are associated. For example, genes 29 and 7 are evolving from a common ancestor of *tps1*. This result was unexpected. However, the tree compared similar amino acids rather than just the percent that were identical. Consequently, the tree shows the similarity in sequences of genes 7 and 29 with *tps1*. Perhaps this suggests that there is a great deal of evolutionary pressure on these sequences to remain similar because they produce similar compounds. These genes may be responsible for the increase in CS that is seen with the addition of volicitin.

Gene 22 had an *E* value of zero with a GcpE protein from tomato, meaning that the protein sequence encoded by the gene is nearly identical to the sequence in tomato. The GcpE protein is involved in the synthesis of *DMADP* and *IPP* in the plastid (Querol et al., 2002). This suggests that the ultimate product of this reaction would be monoterpenes or diterpenes. The oxygenated monoterpene linalool is a predominant component of the volatile mixture. While the RNA from this gene was induced by volicitin, it was unaffected by BAW

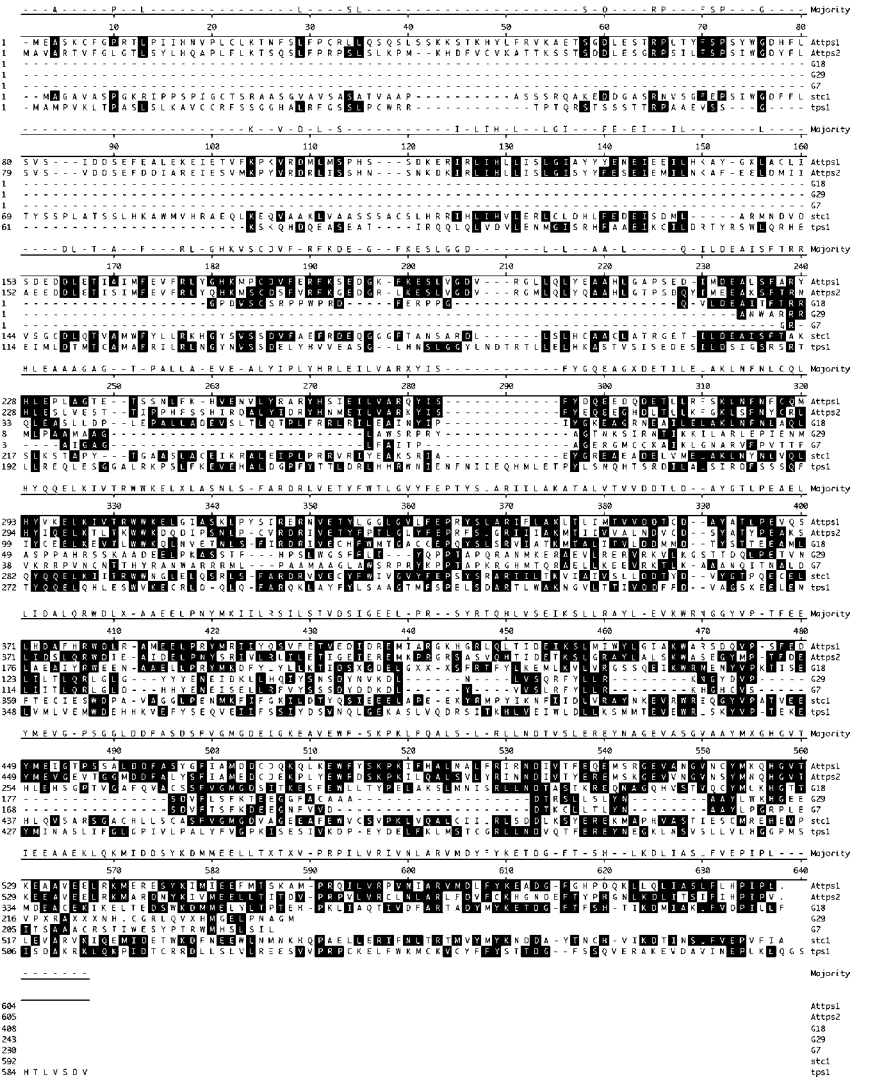

FIG. 3. Alignment of genes 7, 18, and 29 from Table 1 with other sesquiterpene cyclase genes shows similarity of these genes. *Attps1*, *Arabidopsis* At3G29410; *Attps2*, *Arabidopsis* At3G32030; *stc1* and *tsp1*, *Z. mays* sesquiterpene cyclase and terpene synthase.

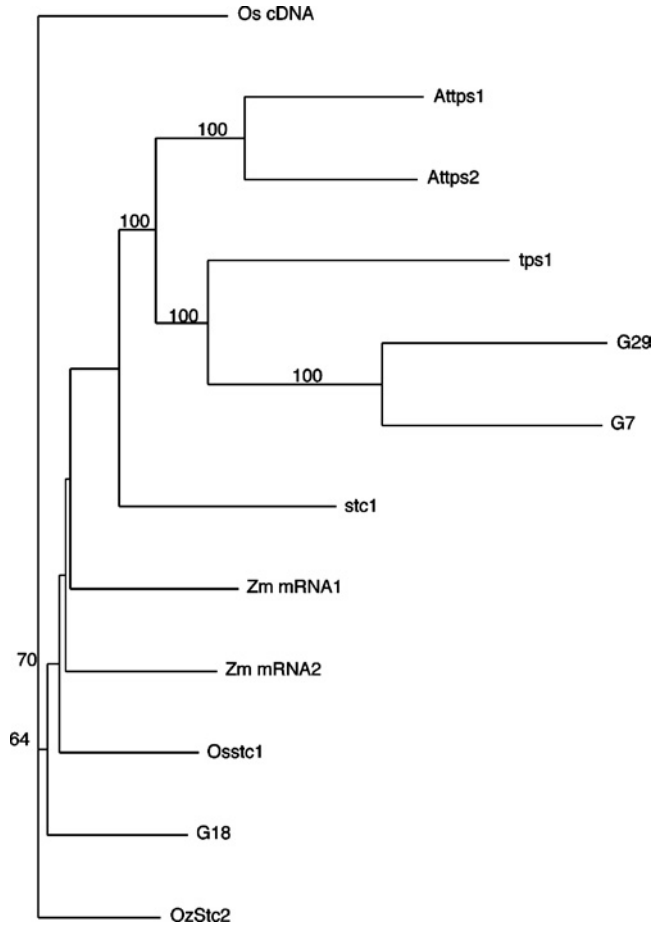

FIG. 4. Phylogenetic tree of most similar sequences with genes 7, 18, and 29 shows evolutionary relationship of these genes. Neighbor joining and distances program of PAUP was used for phylogenetic analysis. Bootstrap analysis (a measure of the percent reliability that the branches are associated) is shown at the nodes. OscDNA, *Oryza sativa* AK108761; *Attps1*, *Arabidopsis thaliana* At3G29410; *Attps2*, *Arabidopsis thaliana* At3G32030; *tps1*, *Z. mays* AAO18435; *stc1*, *Z. mays* AAK73113; maize mRNA1, *Z. mays* PC0101634; maize mRNA2, *Z. mays* PC0100777; Osstc1, *Oryza sativa* BAB63870; Osstc2, *Oryza sativa* BAC99549.1

feeding (Figure 2); additional components of BAW saliva may be responsible for this result. Gene 16 was also involved with volatile production. It is similar to *S*-adenosyl-L-methionine:salicylic acid carboxyl methyltransferase (SAMT). This enzyme is responsible for the conversion of salicylic acid to methyl salicylate, a volatile compound that is responsible for chemoattraction of moths during pollination (Zubieta et al., 2003). However, a role in defense signaling cannot be ruled out since this substance plays a role in inter- and intra plant communication upon pathogen damage. This transcript of this gene was induced by both volicitin and slightly induced by BAW infestation (Figure 2). Considering that other pathogen-associated genes are inhibited by BAW feeding, this suggests that this gene may be involved in chemoattraction.

Volicitin-induced genes identified here are not specifically induced by volicitin or BAW feeding since they are also present in wounded tissue. The only transcripts that were found to be induced by both volicitin and BAW feeding were genes associated with volatile production. Perhaps, this is an indication of the specificity of the genes in the interaction with BAW. This suggests that a complex promoter might be present in these genes, since they are induced by both wounding and volicitin. Our finding of genes that are induced by volicitin but inhibited by BAW infestation was surprising, and suggests an added complexity to the promoters of these genes. An additional factor found in BAW/maize interaction is suggested by this result. The inhibition or lack of response of genes by BAW that are in turn induced by volicitin points to the idea that the plant is evolving in response to a complex mixture in the regurgitant. Consequently, for some genes, an induction by volicitin does not mimic the plant's interaction with BAW. In the plant's response to insect attack, the inhibition of some genes supercedes the effect of volicitin. Given that the induction of maize genes involved in volatile production is an advantage to the plant, it is unclear what volicitin confers for BAW. However, induction of plant defense genes due to a specific component of the pathogen is observed in plant/pathogen interactions.

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